

JUVENILE NEMATODES (*ECHINOCEPHALUS PSEUDOUNCINATUS*)  
IN THE GONADS OF SEA URCHINS (*CENTROSTEPHANUS*  
*CORONATUS*) AND THEIR EFFECT ON HOST GAMETOGENESIS

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Although sea urchins have been used as biological research material for many years, particularly as a source of gametes, there have been few reports of infection of these animals by nematodes. The nematode *Echinomermella grayi* (Gemmill and von Linstow, 1902) tentatively placed in the Merminithoidea by Chitwood (1933), is known from a single specimen found in the perivisceral coelom of *Echinus esculentus* off Britain, and perhaps a second specimen (Irving, 1910; Ritchie, 1910). Juvenile specimens of gnathostomatid nematodes have been reported twice from sea urchins: *Echinocephalus uncinatus* "perhaps accidentally" in tropical sea urchins (Shipley and Hornell, 1904) and a single specimen of *E. pseudouncinatus* in the gonads of *Arbacia punctulata* at Woods Hole, Massachusetts (Hopkins, 1935; Millemann, 1951). Nematodes have also been found in the gonads of the urchin *Astropyga pulvinata* off Acapulco, Mexico, but these have not been identified (J. S. Pearse, unpublished observations). Neither Hyman (1951, 1955), Johnson (1968), Johnson and Chapman (1969), nor Holland and Holland (1970) cite any other record of nematodes in echinoids.

We report herein the regular occurrence of juvenile *Echinocephalus pseudouncinatus* in the gonads of *Centrostephanus coronatus* off Southern California. Between about 40 and 80% of the sea urchins contained the nematodes in their gonads. Moreover, gametogenesis in parts of the gonads was profoundly affected by the presence of the nematodes.

MATERIALS AND METHODS

Most specimens of *Centrostephanus coronatus* were collected from 3 to 10 m depth by scuba diving off the northeastern shore of Big Fisherman's Cove, Santa Catalina Island, near the Santa Catalina Marine Biological Laboratory. Other samples were taken from Pin Rock in Catalina Harbor on the opposite side of Santa Catalina Island, and from Whistler's Reef off the mainland coast of California near Corona del Mar. These three areas are all in quite different waters: Big Fisherman's Cove is on the Santa Catalina Channel side of Santa Catalina Island; Catalina Harbor, although less than 3 km distant, is on the Pacific side of the island; and Whistler's Reef is about 25 km away on the opposite side of Santa Catalina Channel. Moreover, one sample of gonads from *C. coronatus* was taken on 26 November 1968 from Bahía Tortola (Turtle Bay) on the west-central coast of Baja California, Mexico.

The sea urchins were lodged deep in crevices among the rocks during the day and were extracted with the aid of a bent metal rod. During the night, animals foraged in the open and were more easily collected.

The specimens were dissected within a day after collection and all the gonads were carefully inspected for the presence of nematodes. Records were kept of the presence or absence of worms in the gonads, with notation of their relative abundance.

Gonads with nematodes were fixed in Bouin's solution or warm formalin-alcohol-acetic acid-water solution (FAA: 1:5:1:4). Some of the fixed specimens were dehydrated in an acetone series, cleared in benzene, embedded in paraffin, sectioned at 10  $\mu$ , and stained in hematoxylin and eosin. Others were dissected whole from the gonads.

TABLE I  
*Incidence of specimens of Centrostephanus infected with Echinocephalus*

Date	Number of <i>Centrostephanus</i>	Number (and per cent) infected with <i>Echinocephalus</i>
Big Fisherman's Cove, Santa Catalina Island		
11 Aug. 69	38	26 (68.5)
20 Aug. 69	7	5 (71.5)
26 Aug. 69	18	14 (78.0)
31 Aug. 69	17	14 (82.5)
15 Oct. 69	10	9 (90.0)
29 Nov. 69	20	16 (80.0)
2 Feb. 70	18	13 (65.0)
9 Mar. 70	25	21 (80.0)
Catalina Harbor, Santa Catalina Island		
28 Aug. 69	22	9 (41.0)
Whistler's Reef, Corona del Mar		
10 Nov. 69	20	8 (40.0)
20 Nov. 69	18	7 (39.0)

#### OBSERVATIONS

##### *Frequency of nematode infection in Centrostephanus coronatus*

A total of 153 specimens of *C. coronatus* were collected from Big Fisherman's Cove, Santa Catalina Island, and examined for the presence of nematodes in their gonads. Of these, 118 (78%) were infected (Table I). The percentage infected in each sample ranged from 65 to 90%. All of the urchins in the August samples were sexed by microscopic examination of gonadal smears; there were 46 males and 34 females, and 31 and 28 were infected, respectively.

All the samples of *C. coronatus* from both Pin Rock in Catalina Harbor and Whistler's Reef off Corona del Mar had lower incidences of infection than those from Big Fisherman's Cove (Table I). Moreover, not only were a smaller percentage of animals infected, but those that were contained fewer nematodes. Most of the infected urchins in Big Fisherman's Cove had multiple infections, with several nematodes in each of the five gonads. In contrast, most of the

infected urchins from both Pin Rock and Whistler's Reef had only one or two nematodes in only one or two of the gonads, and detection of the infection required careful dissection of all the gonads.

Seventeen specimens of *C. coronatus* were also collected and dissected on 26 November 1968 from Bahia Tortola (Turtle Bay) on the west-central coast of Baja California. At the time of the collection, the presence of nematodes was not known, but during the dissection, one gonad was found with an encysted nematode.

#### *The juvenile nematode*

The nematodes we found in the gonads of *C. coronatus* are all juveniles. They do not differ in any respect from Millemann's (1951) description of *Echinocephalus pseudouncinatus*. The most significant feature for identification is the structure of the head bulb (Fig. 1A), which bears 6 rows of about 40 hooks each, with a lateral separation of the hooks and smaller hooks on the dorsal and ventral areas. Figure 1B shows the tail, which was not drawn by Millemann (1951).

*Echinocephalus pseudouncinatus* was originally described from numerous juvenile specimens found in the foot of pink abalones *Haliotis corrugata* from San Clemente Island off Southern California (Millemann, 1951). This is the only species of *Echinocephalus* presently known from Southern California. Juveniles of another species of *Echinocephalus*, *E. uncinatus*, are known to infect molluscs elsewhere (Shiple and Hornell, 1904; Baylis and Lane, 1920), and it is likely that *E. pseudouncinatus* juveniles regularly infect both molluscs and *C. coronatus* in Southern California. Among the sea urchins of Southern California, however, juvenile *E. pseudouncinatus* seem to infect *C. coronatus* specifically. Hundreds of specimens of the sea urchins *Strongylocentrotus purpuratus*, *S. franciscanus*, and *Lytechinus anamesus* from Southern California have been dissected during the past year and only a single specimen of *E. pseudouncinatus* has been found in the gonad of one *S. purpuratus* collected near San Diego. The single, remarkable record of what seems to be a juvenile specimen of *E. pseudouncinatus* in the gonad of a specimen of *Arbacia punctulata* at Woods Hole, Massachusetts, should again be noted (Hopkins, 1935; Millemann, 1951).

#### *Possible adult hosts*

Adults of the genus *Echinocephalus* inhabit the intestine, usually the spiral valve, of elasmobranchs (Hyman, 1951; Yamaguti, 1961). The most conspicuous elasmobranch in the vicinity of Big Fisherman's Cove, Santa Catalina Island, is the California horned shark *Heterodontus francisci*. This species is known to feed on sea urchins and its teeth and bones often are colored purple, presumably from an accumulation of sea urchin naphthoquinone pigments (Leighton Taylor, Scripps Institution of Oceanography, personal communication). A similar purple coloration of teeth and bones occurs in the sea otter *Enhydra lutris*, which feeds on sea urchins (Fox, 1953). Other species of horned sharks are known to feed on sea urchins, including *H. phillipi* and *H. galeatus* off southeast Australia, and *H. japonicus* off Japan (Smith, 1942). Moreover, Saville-Kent (1897, page 192) noted the teeth of *H. phillipi* ". . . are not infrequently stained a deep purple, through constant indulgence in a dietary of the commoner purple urchin."

Accordingly, three specimens of California horned sharks were collected in February 1970 from Big Fisherman's Cove, Santa Catalina Island, and the intestinal contents examined. One large female, measuring 76 cm total length,

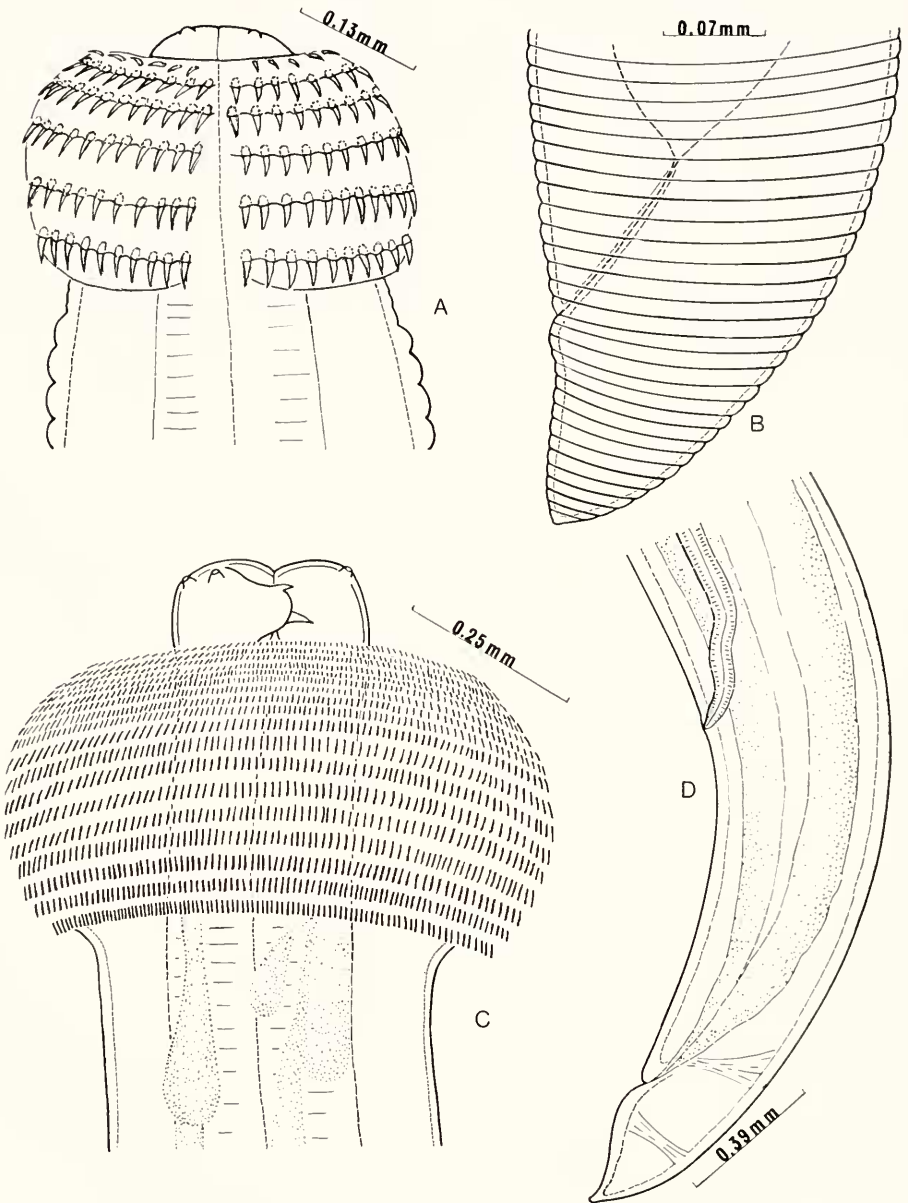


FIGURE 1. Juvenile head, lateral view (A) and juvenile tail (B) of *Echinocephalus pseudo-uncinatus* from the gonad of *Centrostephanus coronatus*, and female head, lateral view (C) and female tail (D) of *Echinocephalus* sp. from the spiral valve of *Heterodontus francisci*.

contained three adult specimens of *Echinocephalus* in its spiral valve. The adult worms, however, have 18 rows of hooks on the head bulbs, each containing 150–200 hooks and therefore, by current taxonomic criteria, cannot be considered the same species as the juveniles in the sea urchin. The number and arrangement of hooks on the head bulb is the same as in *E. southwelli* Baylis and Lane, 1920, but the vulva and cervical sacs are much more anterior than in that species. Since we found no males, no attempt is made to describe the nematode as a new species, but the measurements, a brief description, and drawings (Fig. 1C, D) are given in order that parasitologists might be aware of this previously unknown host-parasite relationship.

Young entire female: Length = 22.46 mm; maximum body diameter = 0.68 mm; esophagus = 3.48 mm; esophagus-vulva = 17.42 mm; vulva-anus = 1.19 mm; tail = 0.37 mm; anal body diameter = 0.26 mm; head bulb =  $0.75 \times 0.44$  mm; lip region =  $0.26 \times 0.12$  mm.

Anterior of one female: Length = 17.42 mm; maximum body diameter = 1.2 mm; head bulb =  $0.93 \times 0.17$  mm; lip region =  $0.44 \times 0.26$  mm.

Posterior of two females: Length = 19.86–24.0 mm; maximum body diameter = 1.34 mm; vulva-anus = 2.75–3.67 mm; tail = 0.58–0.70 mm; anal body diameter = 0.16 mm.

Description: Cuticle sometimes coarsely but irregularly annulated, probably due to contraction, each annulus bearing finer striations. Eighteen rows of hooks on the head bulb, composed of 150–200 hooks each, larger hooks toward posterior; distance between rows less than length of hooks; maximum length of hooks 0.035 mm. Two large lips, each bearing two prominent papillae; overlapping lobe of lip bears two toothlike projections. Cervical sacs extending about  $\frac{1}{2}$  length of esophagus in young female. Deirids not observed. Tail broadly conical, narrowly rounded at tip. These adult nematodes, as well as a representative collection of juvenile *E. pseudouncinatus* from *C. coronatus*, are held in the Nematode Collection of the University of California, Davis.

#### *Nematode effect on urchin gonads*

The echinoid gonad consists of five primary tubules that grow orally along the interambulacra from each of the aborally situated gonopores (Fig. 2). The five primary tubules branch repeatedly so that in adults the five gonads are large organs of intertwining tubules. In *C. coronatus* the gonads are anchor-shaped, with the "flukes" of the anchor situated orally. The gonads are enveloped completely with the thin, ciliated epithelium of the perivisceral coelom, and strands of this epithelium secure the gonad to the inner surface of the test wall and to the gut. Under the perivisceral coelomic epithelium, thin layers of smooth muscle and connective tissue cover the inner germinal epithelium. The germinal epithelium is derived from the hemal strand and consists of both reproductive cells (gonials, spermatocytes or oocytes, spermatozoa or ova) and accessory cells (nutritive phagocytes). Often there is a space ("perihemal coelom") between the perivisceral coelomic epithelium and the muscle layer and another space ("hemal sinus") between the muscle layer and the germinal epithelium.

Young juvenile nematodes were lodged between the perivisceral coelomic epithelium and the germinal epithelium; that is, within the hemal or perihemal

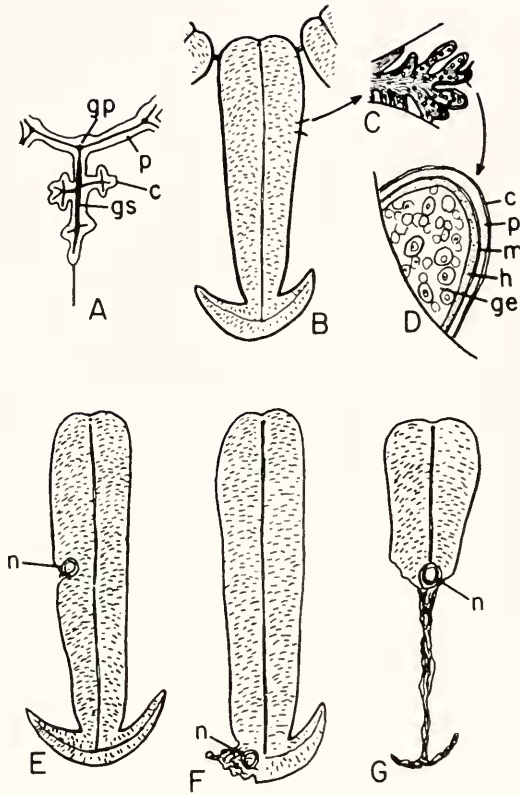


FIGURE 2. Diagrams showing the structure of sea urchin gonads during early development in a juvenile urchin (A) (after Tahara and Okada, 1968), when fully developed in an adult *Centrostephanus coronatus* (B, C, D), and the effect on the gonad when infected by juvenile *Echinoccephalus pseudoincinatus* encysted near the end of a peripheral tubule (E), in the main tubule leading to one of the flukes of the gonad (F), and in the main tubule (gonoduct) near the middle of the gonad (G). All of the gonads are shown from their perivisceral coelomic sides with the oral portion down; abbrev.: c, perivisceral coelomic epithelium; ge, germinal epithelium; gp, position of gonopore; gs, hemal strand; h, "hemal sinus"; m, muscle; n, encysted nematode; p, "perihemal coelom."

spaces of the gonadal wall. Larger worms displaced most of the germinal epithelium in the infected tubule and thick, fibrous connective tissue encysted both the worm and associated germinal epithelium. Cysts with large juveniles protruded into the perivisceral coelom; large juveniles, probably nearly full-grown, broke through the wall of the gonadal cyst and extended free into the coelom.

Although gonadal tubules adjacent to those with the worms usually contained well-developed spermatogenic or oogenic cells, most of the tissue within the cysts in the infected tubules consisted of nongametogenic accessory or connective cells. Moreover, gametogenesis within infected tubules distal (oral) to the worm also seemed suppressed. When the infection occurred near the end of a side tubule, as was usually the case, there was little effect on the overall gametogenic state of the gonad (Fig. 2E). Occasionally, however, infection occurred within a main

tubule and, in such cases, the gonads were shriveled distal to the sites of infection (Fig. 2F, G). In one dramatic example, two worms had lodged in the main tubule (probably the gonoduct) in the middle of one testis; spermatogenesis appeared

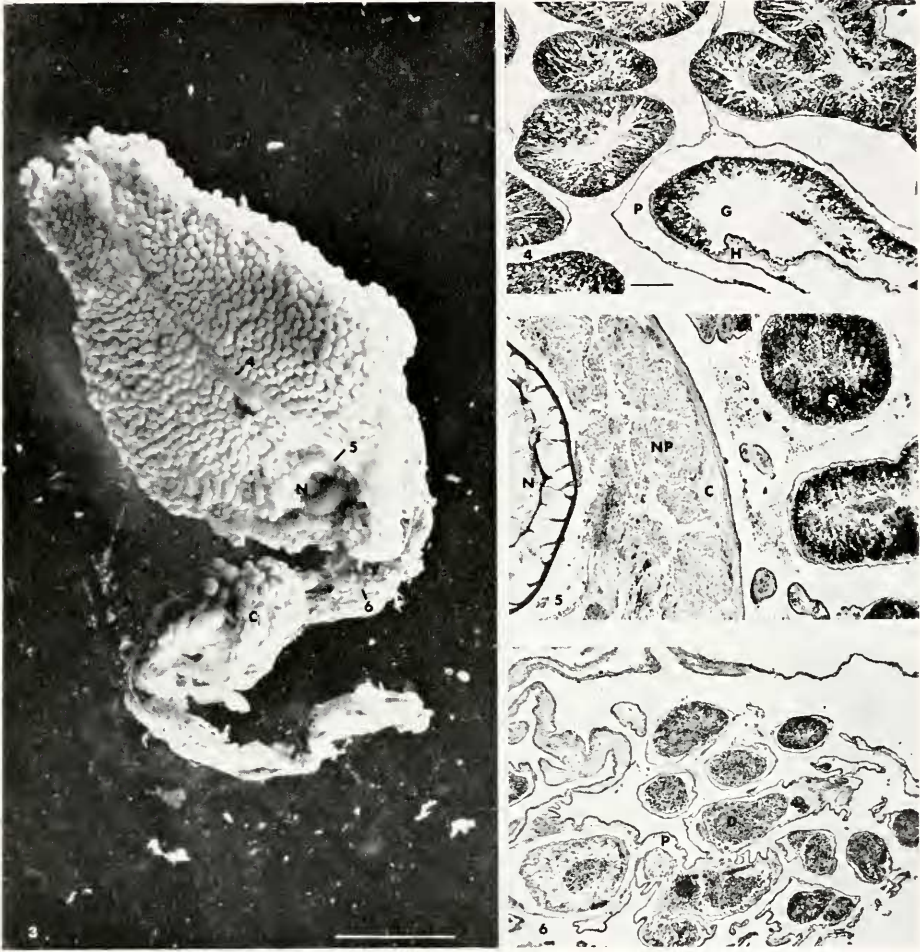


FIGURE 3. A testis of *Centrostephanus coronatus* infected with *Echinocephalus pseudouncinatus* showing a cyst containing one nematode (C) and a second nematode broken partially free of its cyst (N). The oral portion of the gonad is at the bottom of the photograph; note the normal-appearing aboral half of the gonad and the shriveled oral portion. Numbers 4, 5, and 6 correspond to Figures 4 through 6; the scale line indicates 5 mm.

FIGURE 4. Section through the normal-appearing aboral portion of the testis in Figure 3, showing the gonoduct (G) surrounded by perithemal (P) and hemal (H) spaces, and the testicular tubules full of spermatogenic cells (S).

FIGURE 5. Section through the nematode in the testis in Figure 3, showing a portion of the nematode (N) and extensive nutritive phagocytic tissue (NP) in a tubule surrounded by a fibrous cyst wall (C), and adjacent tubules full of spermatogenic cells (S).

FIGURE 6. Section through the shriveled oral portion of the testis in Figure 3 just below the nematode, showing the germinal epithelium full of degenerating cells (D) and large perithemal spaces (P). Figures 4-6 are from 10  $\mu$  paraffin sections stained with hematoxylin and eosin; all are at the same magnification and the scale line in Figure 4 indicates 100  $\mu$ .

active and normal in the aboral half of the testis, while the tubules were shrunken and filled with degradation products orally (Figs. 3-6).

The finding that encysted juvenile worms can suppress urchin gametogenesis has important implications regarding gametogenic control. Suppression does not seem to be due to the release and diffusion of some substance by the worms because gametogenesis is suppressed only oral to the infections rather than all around them. Moreover, the worms are coiled and encased in cysts produced by the urchin gonads; they are not likely to move through the gonads and disrupt gametogenesis. Rather, the encysted worms seem to block the passage of some material that is essential for gametogenesis. Such a gametogenic regulating substance, although never directly demonstrated, is almost certainly present because gametogenesis occurs in synchrony among all five gonads in individual sea urchins (Pearse, 1969). The encysted nematodes probably do not block transport of simple nutrients; radioactive tracing studies have shown that nutrient transfer in urchins occurs mainly through the perivisceral coelom (Farmanfarmaian and Phillips, 1962). Instead, the apparent blockage of gametogenesis by the juvenile nematodes indicates the presence of a hormonal substance which regulates gametogenesis in sea urchins and is transported *within* the gonad (perhaps through the perihemal or hemal spaces) rather than through the perivisceral coelomic fluid.

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#### SUMMARY AND CONCLUSIONS

1. The juvenile phase of the nematode *Echinocephalus pseudouncinatus* occurs commonly in the gonads of the sea urchin *Centrostephanus coronatus* off Southern California. The gonads of about 78% of the urchins in Big Fisherman's Cove, Santa Catalina Island, were heavily infected with the juveniles. About 40% of the urchins at Pin Rock in Catalina Harbor, Santa Catalina Island, and at Whistler's Reef off Corona del Mar on the mainland coast of California were infected, although the infection was usually not as severe as in Big Fisherman's Cove. A juvenile, probably of *E. pseudouncinatus*, also was found in gonads of *C. coronatus* collected from Bahia Tortola off west-central Baja California. Although juveniles of *E. pseudouncinatus* also occur commonly in the foot of pink abalones, they only rarely infect other species of sea urchins in Southern California.

2. The California horned shark *Heterodontus francisci* seems a likely host of the adult phase of *E. pseudouncinatus*. However, when specimens of the horned



shark were examined, adult specimens of *Echinocephalus* were found which do not seem to be the same species as our juvenile specimens.

3. The juvenile nematodes were encysted mainly in the spaces (perihemal or hemal) between the perivisceral coelomic epithelium and the germinal epithelium of the *Centrostephanus* gonad. Large juveniles filled the host gonadal tubule and bulged into the perivisceral coelom. Host gametogenesis was suppressed in the infected gonadal tubule, especially in the oral parts of such tubules. Gametogenesis in adjacent tubules did not seem affected. When the juveniles were in major tubules of the host gonad, severe suppression of host gametogenesis occurred in the oral parts of the gonad. It is suggested that encysted juveniles block the passage through the gonadal tubules of some hormonal substance that regulates urchin gametogenesis.

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